

U.S.S.N. 09/235,875
Filed: January 22, 1999
CLEAN VERSION OF AMENDMENTS PURSUANT TO 37 C.F.R. § 1.121



Clean Version of Amended Claims
Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. (Three times amended) A method for the biological production of polyhydroxyalkanoate containing 3-hydroxyhexanoate comprising growing a transgenic *E. coli* having at least one bacterial transgene encoding a PHA polymerase incorporating C₆ substrates and at least one enzyme selected from the group consisting of a *phbA* thiolase gene encoding an enzyme that converts butyryl-CoA and acetyl CoA to beta-ketohexanoyl-CoA, a *phbB* reductase gene that encodes an enzyme that converts beta-ketohexanoyl-CoA to beta-hydroxyhexanoyl-CoA, a *phbC* polymerase gene that encodes an enzyme that polymerizes 3-hydroxybutyryl CoA, a D-specific enoyl-CoA hydratase and β-hydroxyacyl-ACP-coenzymeA transferase, and providing feedstocks for the transgenic *E. coli*, wherein the production of polyhydroxybutyrate-co-[polyhydroxyvalerate] polyhydroxyhexanoate by the transgenic [organism] *E. coli* occurs.

6. (Twice Amended) The method of claim 1 wherein the *phbC* polymerase gene encoding a PHA polymerase enzyme that incorporates C₆ substrates is incorporated into the bacterial chromosome.

7. (amended) The method of claim 1 wherein the polymerase is from *Aeromonas caviae*, *Comamonas testosteroni*, *Thiocapsia pfenigii*, *Chromatium vinosum*, *Bacillus cereus*, *Nocardia carolina*, *Nocardia salmonicolor*, *Rhodococcus ruber*, *Rhodococcus rhodocrous*, and *Rhodospirillum rubrum*.

8. (amended) The method of claim 1 wherein the transgenic *E. coli* directs metabolites to production of 3-hydroxyhexanoyl-CoA.

ES 9. (Twice Amended) The method of claim 8 wherein the transgenic *E.coli* comprises a *phaJ* transgene encoding a D-specific enoyl-CoA hydratase.

10. (Twice Amended) The method of claim 9 wherein the *phaJ* transgene is isolated from a bacterium selected from the group consisting of *R. eutropha*, *Klebsiella aerogenes*, *P. putida*, and *Aeromonas caviae*.
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11. The method of claim 8 wherein the organism has the genes encoding the enzymes in a butyrate fermentation pathway.

12. The method of claim 11 wherein the enzymes in the butyrate fermentation pathway are from *Clostridium acetobutylicum* or *Thermoanaerobacterium thermosaccharolyticum*.

13. The method of claim 11 wherein the organism converts butyrate to butyryl CoA or butyryl CoA to crotonyl CoA.

14. The method of claim 11 wherein the organism expresses a broad range reductase that is active on C₆ substrates.
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15. (amended) The method of claim 11 wherein the organism expresses a polymerase that accepts 3-hydroxyhexanoyl CoA and 3-hydroxybutyryl CoA.
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16. The method of claim 11 wherein the organism expresses a thiolase accepting acetoacetyl CoA.

17. The method of claim 11 wherein the organism expresses an enzyme selected from the group consisting of thiolases specific for 3-ketohexanoyl CoA, reductase active on 3-ketohexanoyl CoA, and 3-hydroxyhexanoyl CoA.

18. The method of claim 8 wherein the organism expresses one or more fatty acid biosynthetic enzymes.
19. The method of claim 18 wherein the fatty acid biosynthetic enzymes are enzymes converting acyl ACP to acyl CoA.
20. The method of claim 19 where the enzymes are selected from the group consisting of ACP-CoA transacylase, acyl ACP thioesterase, and acyl CoA synthase.
21. The method of claim 20 wherein the enzymes are acyl ACP thioesterase and acyl CoA synthase.
22. The method of claim 18 wherein the enzymes are from *E. coli*.
23. The method of claim 8 wherein the organism expresses one or more enzymes forming a fatty acid oxidation complex.
24. The method of claim 23 wherein the one or more enzymes are selected from the group consisting of enzymes epimerizing S-3 hydroxyhexanoyl CoA and enzymes reducing 3-ketohexanoyl CoA.
25. The method of claim 24 wherein the enzymes are from *Nocardia salmonicolor*.
26. The method of claim 24 wherein the enzymes epimerizing S-3 hydroxyhexanoyl CoA are from the *Pseudomonas putida* FaoAB complex.
27. The method of claim 23 wherein the organism accumulates 3-ketohexanoyl CoA due to a lack of a thiolase.

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31. (amended) A transgenic bacterium or plant for use in any of the methods of claims 1 and 7-30.

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